



PATENT  
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Applicant: James M. Robl et al. Confirmation No.: 4828  
Serial No.: 10/705,519 Art Unit: 1632  
Filed: November 10, 2003 Examiner: Deborah Crouch  
Customer No.: 21559  
Title: TRANSGENIC UNGULATES HAVING REDUCED PRION PROTEIN ACTIVITY AND USES THEREOF

DECLARATION OF DR. JAMES M. ROBL TRAVERSING GROUNDS OF  
REJECTION OVER GOOD

Under 37 C.F.R. § 1.132 and regarding the rejection of claims 1-6, 25-32, and 35-38 for anticipation by Good et al. (U.S. Patent Publication No. 2002/0069423; hereafter “Good”), I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application. My curriculum vita is attached.
2. In my opinion, if one skilled in the art followed the methods outlined in the Detailed Description (including the Examples) of Good, it would not reproducibly result in the production of a prion protein (PrP) knockout cell or bovine.

Good provides a single, prophetic method for producing PrP knockout cows (Example 2). In this proposed method, Good describes a first targeting step in paragraph 165, in which bovine fetal fibroblasts (BFF) are electroporated in the presence of a targeting vector and then plated in 100 mm<sup>2</sup> culture dishes at a density of 500,000 cells per dish. Good proposes culturing the cells in selection

media until colonies form and then isolating the colonies using cloning rings (¶¶ 167 and 169).

In my opinion, such a procedure would not be reproducibly successful. First, as noted by Good, the transfection procedure will produce many neomycin resistant cells, but only a fraction of those cells will have been correctly targeted (¶ 109). As a result, the plating density employed (500,000 cells/dish) is too high to allow for efficient isolation of individual colonies, as the plated cells would be essentially confluent. In addition, fibroblasts are motile cells that migrate in culture dishes, further complicating the isolation of any individual colonies under the conditions proposed in the Good reference. Because the cells are motile and would be essentially confluent, colonies isolated using cloning rings, as taught in Good, would be mixed, i.e., the colonies would contain targeted and non-targeted cells. Southern blot genotyping on such a mixed colony could produce a positive signal if some correctly targeted cells were present. There would, however, be no guarantee that fetuses produced using cells from this mixed colony would be transgenic, as a large proportion of the cells would not harbor a prion knockout.

3. Moreover, Good's proposed primary screening method for identifying correctly targeted prion knockout cells would also be incompatible with the use of fibroblasts, which senesce. As described in paragraph 169, Good proposes duplicating each colony and then performing PCR followed by Southern blotting for genotyping. Good, however, provides no teachings on how to obtain sufficient DNA from selected colonies to perform PCR followed by Southern blotting. As noted by Good, the proposed cloning process requires many population doublings, leaving few doublings left for expansion (¶ 171). In my opinion, the targeted cells would senesce before a sufficient quantity of DNA was obtained to perform the proposed genotyping step. No alternative screening procedures are disclosed.

Good also proposes expanding the colonies for freezing but fails to provide any methods for carrying out such a process. Because the cells in Good are near senescence, it would be difficult or impossible to produce adequate cells for freezing as the cells in Good's technique would not be dividing.

4. Similarly, Good provides insufficient guidance for the second targeting step, which would be necessary to produce homozygous KO cows. As noted by Good, a second round of targeting is typically inefficient because the second vector often recombines with the previously targeted allele and the use of the same selection marker will not distinguish between heterozygous and homozygous cells (¶ 206). While Good does suggest use of a second marker to determine whether the second vector has been successfully incorporated into a cell, Good fails to provide any guidance on how to prevent retargeting of the first allele. In these experiments, retargeting of the first allele occurs frequently, resulting in a failure to produce homozygous knockouts or requiring an impractical number of transfections.

5. The processes described in the present application used to successfully produce a homozygous prion knockout bovine are distinct from those of Good. To the best of my knowledge, we are the first to produce PrP knockout bovines. The specification describes the identification of a genomic PrP DNA fragment (page 46, lines 11-17 and page 55, lines 13-20), provides exemplary targeting vectors and methods for their construction (page 46, lines 17-30, page 47, lines 12-23, and page 55, lines 20-30), gives structural information on the vector and methods of animal cloning (page 55, line 30 – page 56, line 26 and page 58, line 28 – page 59, line 19), and provides a diagnostic PCR to genotype the cells following drug selection (page 56, line 28 – page 57, line 25 and page 59, line 21 – page 61, line 17). Furthermore, the specification provides data showing that hemizygous and

homozygous KO cells were actually produced using the described methods (Example 1, pages 54-61).

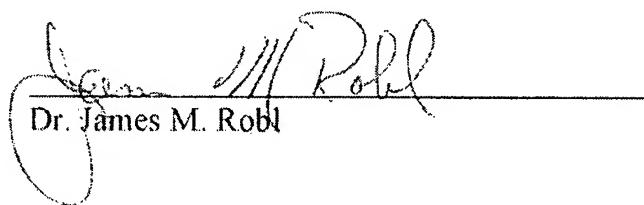
In particular contrast to Good, the specification teaches dilution plating of 10,000,000 electroporated cells into sixty 24-well plates, resulting in a much lower initial density of cells (~7000 per well) compared to Good (~500,000 per well) and allowing the formation of individual colonies in a well (page 56, lines 5-26). This procedure allows for isolation of a colony of homogenous cells.

In addition, the specification provides a PCR-based screening method for targeted integrations that does not rely on Southern blotting. This screening method employs two sets of primers for targeting of one allele (F7 and R7 and F10 and R10) and one set of primers for targeting of the other allele (F14 and R14) followed by electrophoretic analysis of the PCR products (page 56, line 28 to page 57, line 25 and page 59, line 21 to page 60, line 18). With these two different PCR primers, we could distinguish a homozygous KO from a hemizygous KO. Taking the low frequency of homozygous KO in somatic cells and animal cloning into consideration, such a checkpoint step for isolation of homozygous KO cells from hemizygous KO cells is important for a preparation of a homozygous KO bovine. Furthermore, these methods require minimal population doublings to produce sufficient DNA, unlike the Southern blotting approach of Good.

In further contrast to Good, the specification illustrates in Figs. 44A and 44B two prion protein knockout vectors used to produce homozygous knockout cells. A vector having a neomycin resistance gene was employed to form heterozygous PrP KO cells, and a vector having a puromycin resistance gene was employed to form homozygous PrP KO cells. Experimental evidence shows that, when the neo vector was employed first, the neo vector preferentially targeted one allele, and the puro vector preferentially targeted the other.

6. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

31 May 2007  
Date

  
Dr. James M. Robl